Synthesis and Characterization of Bioplastic Film Produced from Human Hair Keratin and Study of its Degradation Pattern by Keratinophilic Fungi

A SYNOPSIS OF RESEARCH WORK PROPOSED TO BE CARRIED OUT
IN PURSUANCE OF THE REQUIREMENT FOR
THE AWARD OF THE DEGREE
OF
DOCTOR OF PHILOSOPHY
IN
BOTANY

Submitted By

SHUBHA DHAWAN

Under the Supervision of

Dr. SHARMITA GUPTA

DEPARTMENT OF BOTANY, FACULTY OF SCIENCE
DAYALBAGH EDUCATIONAL INSTITUTE (DEEMED TO BE UNIVERSITY)
DAYALBAGH, AGRA – 282005

2020

Introduction

Plastics are the material consisting of synthetic or semi-synthetic organic compounds that can be molded into variety of shapes and solid objects. The word Plastic comes from the Greek word Plastikos that means “capable of being shaped or molded”. The plasticity or malleability of the material depicts its ability to go through various processes like casting, Pressing, Molding, Extruding during the manufacture process of different forms like films, sheets, fibers, plates, tubes, bottles, boxes, bags and many more. Majority of plastics are formed from long chains of monomers containing carbon, and thus are regarded as organic polymers with several thousands of repeating units. The properties of the plastics can be modified by the addition of different molecular groups that are hung from the back bone of carbon chain.

Based on the permanence or impermanence of the plastic form, these can broadly be classified as Thermoplastic or thermosetting polymers. Thermoplastics are the group of plastics that can be molded again and again as upon heating they do not undergo any chemical change in their composition. Examples of thermoplastic include Polyethylene (PE), Polypropylene (PP), Polyvinyl Chloride (PVC) and Polystyrene (PS). On the other hand thermosetting polymers can be molded just once, once they have solidified, they stay solid. In the thermosetting process the chemical change occurring is irreversible.

The most commonly utilized Polyethylene plastics are the range of inexpensive plastics that are used in the supermarket bags, bottles and other day to day single use items. The Polyethylene based plastics can further be of three types – HDPE (High density Polyethylene), LDPE (Low density polyethylene) and Polythylene tetraphalate(PET).

HDPE are used in commodities like detergent bottles, milk jugs and others. The LDPE plastics find uses in such as outdoor furniture, shower curtains, plastic parts of computer components, wash bottles etc. The excellent moisture resistant property of Polyethylene tetraphathalate makes it suitable for use as food packaging material; hard cosmetic jars microwavable containers and transparent films.

In 2018, the global production of plastics reached 359 million metric tons, with 62 million metric tons produced in Europe alone. (Fig 1) China is one of the largest producers of plastics in the world, accounting for more than one quarter of the global production.

According to a report on Indian Plastic Industry by PlastIndia Foundation,2018 there was a total consumption of 14550 KT of thermoplastics and 950 KT of Thermosets in the year 2016/2017 in India alone[19].

Microdebris –

These are the plastic pieces between 2mm and 5mm, also known as nurdles. The microdebris reaches the ocean water through rivers and streams and these are consumed by filter feeding organisms. A 2004 study by Richard Thompson from the University of Plymouth, UK, found a great amount of microdebris on the beaches and waters in Europe, the Americas, Australia, Africa, and Antarctica. If not ingested, this microdebris floats instead of being absorbed into the marine environment.

Macrodebris -

Plastic debris is categorized as macrodebris when it is larger than 20 mm. These include items such as plastic grocery bags. Macrodebris are often found in ocean waters, and can have a serious impact on the native organisms. Fishing nets have been prime pollutants. Even after they have been abandoned, they continue to trap marine organisms and other plastic debris. Eventually, these abandoned nets become too difficult to remove from the water because they become too heavy, having grown in weight up to 6 tons.

In 2019 a new report "Plastic and Climate" was published. According to the report, in 2019, plastic will contribute greenhouse gases in the equivalent of 850 million tons of carbon dioxide (CO_2) to the atmosphere.

The excessive use of plastic has brought us in a situation where everyone has a slogan to express “Say No to Plastic!! “ and the concerned bodies or environmentalist are trying continuously to come up with solutions in the form of Bio based plastics made up from natural/ renewable sources.

Bioplastics

Bioplastics are the plastic material made up from natural resources that consist of renewable biomass including vegetable fats and oils, starch from different vegetable sources, straw woodchips, proteins from animal and plant sources and others. Bioplastics are currently being used for disposable items such as crockery, cutlery, pots, bowls, shopping bags.

50 percent of bioplastic market share is currently represented by starch based thermoplastics. Plasticizers such a glycerol are added to starch so that it can be processed thermo – physically. Blends of starch with Polylactic acid, polycaprolactone, EcoFlex (polybutylene adipate-co-terephthalate produced by BASF) are produced industrially and are also composatable. Starch/ polyolefin blends are produced industrially which are not biodegradable, but have lower carbon footprint than petroleum based plastics.

Starch based plastics are complex blends of starch with compostable plastics such as Polylactic acid, Polybutylene Adipate Terephthalate, Polybutylene Succinate, Polycaprolactone, and Polyhydroxyalkanoates. These complex blends improve water resistance as well as processing and mechanical properties. On the other hand Cellulose based bioplastics are made up of cellulose esters and their derivatives. Cellulose acetate is thermoplastic but is expensive and therefore rarely used in packaging.

Various proteins from animal and plant origin are being studied to make bio based plastics. Proteins like casein, wheat gluten and keratin have the promising properties to be used as raw material for different biodegradable polymers. Soy protein based plastics can be considered as another source for the production of Bioplastics but they have disadvantages such as water sensitivity and relatively high cost. Proteins from animal origin researched for the production of bioplastics are blood meal, Egg albumin, feather meal, leather scrap and microbial sludge.

Keratin

Keratin belongs to the group of fibrous structural proteins. It makes up the structures in living systems such as hair, nails, feathers, horns and claws. Keratin as a protein also prevents the epithelial cells from stress or damage.

Most eukaryotic cells contain in their cytoplasm a more or less elaborated cytoskeletal system consisting of intermediate filaments (IF), which are chemically very stable long and unbranched filaments of approximately 10 nm in diameter. Among the various families and subfamilies of IF proteins, that of the keratins is outstanding due to its high molecular diversity.Keratin studies started with sheep hair by Crick in 1952, Powell and Rogers in 1986, Oshima in 2007. Steinert et al.

discovered that the denatured, soluble proteins isolated by dialysis in vitro could spontaneously self – assemble and polymerize to form keratin filaments [24].

No report is available regarding human hair keratin for the formation of bioplastic film except chicken feather [1] and sheep wool [10]. Therefore present research work will be focused on the production of bioplastic film from human hair and study of its degradation pattern by keratinophilic fungi.

Review of literature

Many researchers have reported the utility of keratin protein as the source of bioplastic film production. The major sources of keratin used are chicken feather and sheep wool. The blends of keratin with PLA, cassava starch and cellulose have also been reported. Extrusion studies, tensile properties and biodegradation studies of keratin and keratin blends based bioplastic films have been studied by various researchers. The following table presents the review on such studies:

S.No.	Title	Major Findings	References
[1]	**Keratin based bioplastic film from chicken feathers and its characterization**	• Glycerol at increasing concentrations decreased the maximum tensile strength of chicken feather keratin films. • Bioplastic made up from keratin with 2 wt% of glycerol has the best mechanical and thermal properties. • Bioplastic produced are proven as a biodegradable.	**Ramakrishnan et al.** Keratin based bioplastic film from chicken feathers and its characterization, International Journal of Biological Macromolecules, 2018.
[2]	**Extrusion of Feather Keratin**	• Keratin obtained from poultry feathers was extruded at 120°C using a combination of glycerol, water, and sodium sulfite as processing aids. • The lowest viscosity blends at a constant feather keratin concentration of 60 wt % were found at glycerol concentrations that were higher than the water concentration and sodium sulfite concentrations of 3– 4 wt % of the feather keratin fraction.	**Barone et al**.: Extrusion of Feather Keratin, Wiley InterScience, 2005.
[3]	**The structure, tensile properties and water resistance of hydrolyzed feather keratin-based bioplastics**	• The thermal analysis showed that high temperature and high pressure improved the compatibility between the glycerol and the HFK molecules. • By increasing the glycerol content, the film tensile strength (σb) decreases from 10.5 MPa to 5.7 MPa and the solubility increases from 15.3% to 20.9%, while the elongation at break (εb) achieves the maximum value of 63.8% for the film with 35%	**YaoDou et al.**: The structure, tensile properties and water resistance of hydrolyzed feather keratin-based bioplastics, Chinese Journal of Chemical Engineering, 2016.

		glycerol. • The swelling was just below 16.9% at 25 °C for 24 h, suggesting a good stability of the films in water.	
[4]	**An efficient conversion of waste feather keratin into ecofriendly bioplastic film**	• The keratin powder was used to synthesize a bioplastic flm using glycerol (3.5%) and microcrystalline cellulose (0.2%) in NaOH for 48 h at 60 °C. • The calculated thickness of bioplastic flm was 1.12 × 10−4 mm with tensile strength of 3.62 ± 0.6 MPa. • The Young's modulus and break elongation for synthesized bioplastic flm were 1.52 ± 0.34 MPa and 15.8 ± 2.2%, respectively	**Sharma et al.**: An efficient conversion of waste feather keratin into ecofriendly bioplastic film, Clean Technologies and Environmental Policy, 2018.
[5]	**Biodegradability of injection molded bioplastic pots containing polylactic acid and poultry feather fiber**	• Biodegradability of pot type A (composed of 100% polylactic acid (PLA)) was very low (13 ± 3%) compared to literature values for other PLA materials. • Biodegradability of pot types B (containing 5% poultry feather, 80% PLA, 15% starch), and C (containing 50% poultry feather, 25% urea, 25% glycerol), were 53 ± 2% and 39 ± 3%, respectively. • More than 85% of the total biodegradation of these bioplastics occurred within 38 days.	**Ahn et al.**: Biodegradability of injection molded bioplastic pots containing polylactic acid and poultry feather fiber, Bioresource Technology,2011.
[6]	**Physicochemical properties of cassava starch and starch-keratin prepared biofilm**	• This study investigates the properties of biofilms prepared using starch/keratin blend with and without formaldehyde. • From the water testing results, thickness swelling, water absorption capacity and linear expansion of biofilm without formaldehyde after 10 s of soaking in water were 28.59%, 8.89% and 4.90% respectively and 65.30%, 91.33% and 46.29% respectively after 40 s.	**Oladayo et al.**: Physicochemical properties of cassava starch and starch-keratin prepared biofilm, Songklanakarin J. Sci. Technology, 2016.
[7]	**Partially Carboxymethylated Feather Keratins. 2. Thermal and Mechanical Pproperties of Films**	• Free cysteine thiol groups of keratin extracted from chicken feathers were partially carboxymethylated with iodoacetic acid (25-76% cysteine modification). Stable dispersions were used for the preparation of films by solution casting. • The degree of crystallinity in the films was higher when more cysteine residues were carboxymethylated. The films displayed an optimum in mechanical properties at ~50%	**Schrooyen et al.**: Partially Carboxymethylated Feather Keratins. 2. Thermal and Mechanical Properties of Films, J. Agric. Food Chem, 2001.

		cysteine carboxymethylation.	
[8]	**Poly(ε-caprolactone)/keratin-based composite nanofibers for biomedical applications**	• Keratin-based composite nanofibers have been fabricated by an electrospinning technique. • Aqueous soluble keratin extracted from human hair was successfully blended with poly(ε-caprolactone) (PCL) in different ratios and transformed into nanofibrous membranes. • Nanofibrous membranes with PCL/keratin ratios from 100/00 to 70/30 showed good uniformity in fiber morphology	**Edwards et al.**: Poly(ε-caprolactone)/keratin-based composite nanofibers for biomedical applications, Journal of Biomedical Material Research, 2015.
[9]	**Injection Molded Biocomposites from Soy Protein Based Bioplastic and Short Industrial Hemp Fiber**	• Soy protein based biodegradable plastics as matrix materials and industrial hemp fiber as reinforcing material have been successfully fabricated using the industrial processing techniques of extrusion and injection molding • There was a significant improvement in mechanical properties including tensile properties, flexural properties and impact strength, as well as the heat deflection temperature with the addition of hemp natural fiber to the soy flour bioplastics.	**Mohanty et al.**: Injection Molded Biocomposites from Soy Protein Based Bioplastic and Short Industrial Hemp Fiber, Journal of Polymers and the Environment, 2005.
[10]	**Tough and Functional Cross-linked Bioplastics from Sheep Wool Keratin**	• Novel bioplastic flms derived from wool keratins were prepared by protein solution in an alkaline mild oxidative method that splits disulphide (-S-S-) bonds. • Keratin flms were plasticised with glycerol and sodium dodecyl sulphonic acid (SDS), which provided diferent hydrophobic character to bioplastics • The spectroscopic analyses revelled that these bioplastic flms absorb UV light, what is interesting for packaging applications. • Thermogravimetric and thermomechanical analysis revealed high stability of keratin macromolecules up to 200°C with no inherent thermal transitions. • Tough bioplastics (19±4 MJ· m−3) were obtained after thermal cross-linking with glycerol and formaldehyde outperforming mechanical properties previously reported for protein flms.	**Borja et al**: Tough and Functional Cross-linked Bioplastics from Sheep Wool Keratin, Scientific Reports.
[11]	**Improved**	• bioplastic films were developed using	**Alashwal et al.**:

	properties of keratin-based bioplastic film blended with microcrystalline cellulose: A comparative analysis	the different ratio of keratin extracted from chicken feathers and cellulose. • Although the conducted analysis showed excellent characteristics of bioplastic (K-60), the desired improvement was noticed in the presence of cellulose. • FTIR spectra showed that the absorbent peaks appeared in the films indicating that they sustain the macromolecular structure of the chicken feather keratin, extracted and there are possible hydrogen bonds between keratin protein and cellulose.	Improved properties of keratin-based bioplastic film blended with microcrystalline cellulose: A comparative analysis, Journal of King Saud University – Science,2019
[12]	**Bioplastic based on 1,8-octanediol-plasticized feather keratin: A material for food packaging and biomedical applications**	• 1,8-Octanediol (OD) was used as a plasticizer to modify keratin films in this study. • Two keratins with different structures were extracted from duck feather, including reduced keratin (RK) and native keratin (NK). • The water vapor permeability varied between 0.106 and 0.808 g/(m s Pa) for CK films without OD and with 0.30 g OD/g keratin, respectively, and the tensile strength decreased from 12.1 to 8.0 MPa	**Liu et al.**: Bioplastic based on 1,8-octanediol-plasticized feather keratin: A material for food packaging and biomedical applications, Inc. J. Appl. Polym. Sci., 2018.
[13]	**Utilization of Lime Juice to Enhance Mechanical Performance of Sago-Starch Based Biofilm Reinforced Chicken Feather Keratin**	• This research utilized natural resources, i.e., chicken feather keratin to reinforce sago-starch biodegradable film with glycerol as plasticizer and green lime juice's citric acid as cross-linker. The objective of this research was to observe mechanical performance of biofilm based on several experiments with lime juice concentration, chicken keratin composition and sago-starch variables as independent variables (factors). • The result showed that lime juice can effectively improve the mechanical performance of the biofilm.	**Silviana et al.**: Utilization of Lime Juice to Enhance Mechanical Performance of Sago-Starch Based Biofilm Reinforced Chicken Feather Keratin, Advanced Science Letters, 2017.
[14]	**Keratin-Based Biotechnological Applications**	• Wool, feather, horn and hooves, etc., are strong in terms of mechanical strength due to the presence of keratin. But once keratin is extracted from natural sources then, it becomes poor in mechanical properties. • So, the blending of keratin with other biopolymer can improve the material properties like strength, flexibility, and water vapor permeability. • The malleable nature of keratin	**Nayak et al.**: Keratin-Based Biotechnological Applications, Keratin as a Protein Biopolymer,2018.

		proves its biotechnological applications such as tissue engineering scaffold, green composites, green cement, bioplastic, etc.	
[15]	**Improvement of mechanical and water absorption properties of plant protein based bioplastics**	• This study considers potato and rice proteins as a new source for the manufacture of bioplastics. • Compared to well-known wheat gluten, these bioplastics required higher temperatures for their thermomoulding. However, both of them were more structured materials and exhibited less water absorption (e.g. as low as 9 wt.%) than those obtained for wheat gluten blend.	**Gómez-Heincke et al.**: Improvement of mechanical and water absorption properties of plant protein based bioplastics, Food Hydrocolloids, 2017.
[16]	**Characterization of pea protein-based bioplastics processed by injection moulding**	• This study assesses the behaviour of pea protein isolate (PPI) as a potential candidate for the development of biobased plastic materials processed by injection moulding. • Around 30–40% of glycerol as plasticizer was required to obtain good processability of PPI/GL blends to produce bioplastics. A	**Perez et al:** Characterization of pea protein-based bioplastics processed by injection moulding, food and bioproducts processing, 2016.
[17]	**Reinforcement of Soy Protein-Based Bioplastics Through Addition of Lignocellulose and Injection Molding Processing Conditions**	• The aim of this work was to develop soy protein-based bioplastics with lignocellulosic fiber (additive) by injection molding. • In any case, these findings support the role of cellulosic compounds as additives in protein bioplastics.	**Gamero et al.**: Reinforcement of Soy Protein-Based Bioplastics Through Addition of Lignocellulose and Injection Molding Processing Conditions, Journal of Polymers and the Environment, 2019.
[18]	**Protein/glycerol blends and injection-molded bioplastic matrices: Soybean versus egg albumen**	• Soy Protein isolate(SPI) and egg white albumen concentrate (EW) have been utilized to produce bioplastics through injection molding. • When examining their tensile and water uptake properties is found that SPI bioplastics are more ductile and present enhanced water uptake behavior over EW bioplastics.	**Fernández-Espada et al.** : Protein/glycerol blends and injection-molded bioplastic matrices: Soybean versus egg albumen, Journal of Applied Polymer Science, 2016, 133, 42980.

Objectives

1. Isolation of keratin from human hair samples by Shindai method.

2. Formation of Bioplastic film from the keratin derived from human hair with Glycerol as plasticizer.

3. Characterization of keratin bioplastic film produced, by microscopic and XRD techniques.

4. Isolation and Identification of keratinophilic fungi from keratin sources by hair baiting technique.

5. Study of degradation pattern of keratin bioplastic film by keratinophilic fungi.

Hypothesis

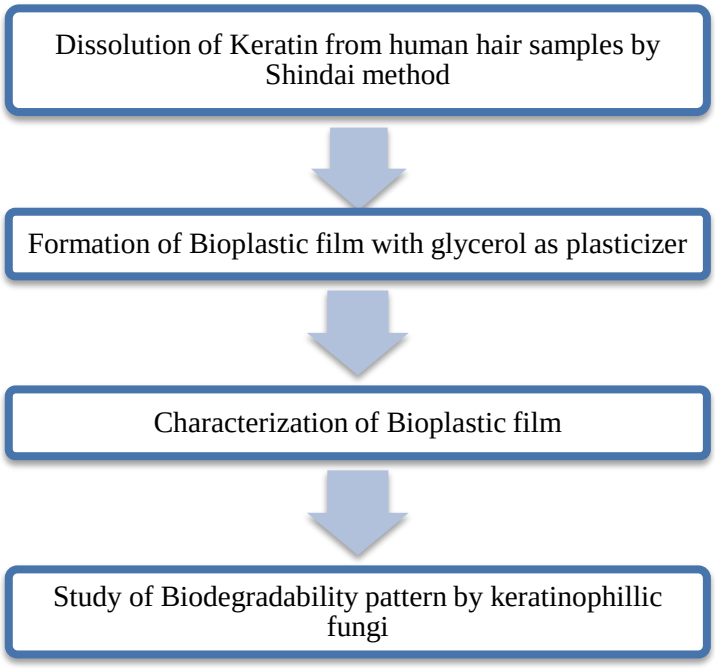
Dissolution of Keratin from human hair samples by Shindai method
Formation of Bioplastic film with glycerol as plasticizer
Characterization of Bioplastic film
Study of Biodegradability pattern by keratinophillic fungi

Figure 1: Flow pattern of proposed research work

The proposed work would be carried out in four phases:

1. First of all, the keratin fraction would be isolated from the human hair protein sample by Shindai method [21].
2. The keratin thus obtained will be utilized to make bioplastic sheets by incorporating glycerol as plasticizer.
3. Thereafter the characterization of the bioplastic film will be done by using techniques such as XRD, SEM and FTIR.
4. Also, biodegradation studies of these bioplastic films will be performed to determine the rate of degradation of these films.

Material and Methods

A**. Collection of hair Sample** – Hair sample would be collected from local salons in Agra, Dayalbagh region in sterile paper bags and stored at 4 °C for further use.

B. **Delipidisation** – Hair sample are washed with double distilled H_2O twice and then soaked in Chloroform and Methanol (2:1) solution overnight. The lipids could be seen as oily layer at the top [23].

C. **Keratin extraction by Shindai method**: Delipidised hair sample are cut into small pieces with the help of blades or scissors and soaked in the extraction buffer. The sample is kept in oven for 3 days at 50 °C [20]. The extracted keratin is separated by filtration and later on centrifugation at 15000 rpm for 20mins.

Buffer Composition –

Sno.	Chemical	Molarity
1	Tris HCl	25mM
2	Thiourea	2.6M
3	Urea	5M
4	Mercaptoethanol	5%

D. **Formation of Bioplastic Film** – The extracted keratin will be dissolved in 2N NaOH, mixed with glycerol and casted as film on the suitable glassware. The resulting film will be dried in oven at 60 °C for 24 h [1].

E. **Characterization methods –** The bioplastic fim formed by the dissolution of human hair keratin will be characterized by following methods [1]:

1. **Scanning Electron Microscope**: Surface characteristics would be analyzed by SEM. This would help us to identify if the mixtures are well mixed during the polymerization process.

2. **Fourier Transform infrared spectroscopy (FTIR**): This technique measures the absorption of infrared radiation by the sample material versus wavelength. The infrared absorption bands identify molecular components and structures. This would help us identify the changes in wave number corresponding to addition of different concentration of plasticizer.

3. X – ray Diffraction (XRD): This method would help us to determine the crystalline structure of the bioplastic film thus formed. Bioplastic film with different concentration of glycerol shall have different crystalline structure and this could be revealed by XRD.

4. Mechanical Property Testing: Tensile strength and Young's modulus of the bioplastic film would be analysed by using the mechanical testing machine.

F. **Isolation and Identification of Keratinophilic Fungi from Soil using Hair as Substrate**: Keratinophilic fungi would be isolated by To – ka –Va hair bating technique of Bendeck (1962) using sterilized human hair as baits [21]. The soil samples will be incubated with hair baits at 28 °C and the visible mycelium appearing on baits will be transferred to petridish containing SDA (Sabouraud's Dextrose Agar Medium ; Agar – 20gms , Dextrose – 40gms, peptone – 10 gms, distilled water – 1000ml). The purified colonies of fungi would be obtained by sub-culturing on SDA medium. The purified culture of keratinophilic fungi would then be identified with the help of literature available in Microbiology lab, Botany Department: Laboratory Manual of Introductory Mycology (Alexopaulus and Beneke 1962), Manual of Soil fungi (Gillman, 1957), Manual of *Chrysosporium* and Allied genera (Van – oorchoot 1980) and A Colour Atlas of Pathogenic fungi (Frey wt al 1986).

The saprophytic keratinophilles will be separated by the perforating and non perforating behavior of keratinophilles . These keratinophilles will be tested by technique proposed by Ajello and George, 1957 [26].

The growth form 14 day old colonies of each strain in Sabouraud's dextrose agar will be used to inoculate hair plates. Autoclaved, short segments (15-20mm) of sterilized human hair will be taken in petriplates and to each petriplate, 20 ml of sterilized distilled water and 3 drops of 10 percent yeast extract will be added aseptically. Several pinhead size fragments from 14 days old colony of each test fungi will be inoculated onto hair in each Petriplates. The plates will be incubated at 28°C in dark and will be examined at intervals of 5 days, upto 30 days.

G. **Degradation of keratin bioplastic film by isolated fungi**.

The isolated and identified fungal culture will be utilized to study the biodegradation of the bioplastic films. The studies will be conducted in 250ml Erlemmeyer flasks containing 50 ml of basal solution ((g/l): 0.1, KH_2PO_4; 0.01, $CaCl_2$; 0.1, $FeSO_4$ $7H_2O$; 0.005, $ZnSO_4$ $7H_2O$; pH = 7.5) and 50mg of bioplastic substrate (Mariana Călin et al, 2017). The inoculated flasks would then be incubated on a rotary shaker at 100 rpm and 27 ± 1°C for 21 d. The fungal culture would be filtered by Whatman filter paper No. 1[22]. The bioplastic substrate would be washed gently to remove fungal hyphae, dried at 60 °C for 48 hrs and analyzed.

Significance of Current Research

The bioplastic film thus produced and characterized could be effectively utilized to replace the petroleum based plastics in applications including packaging and manufacturing of items such as bowls, cups, boxes, bags and many other day to day utilities. The keratin based bioplastics being produced in the current study would be biodegradable and hence would therefore help to combat the problems faced due to petroleum based plastic.

Keratin based bioplastics, if industrialized, are expected to be the best outcome derived from the waste. Currently the starch based bioplastics derived from corn, cassava and other sources have the clashing requirements with the nutritional requirements of the society. Also the bioplastics derived from biopolyesters like PHA and PHB involve considerable cost in the downstream processing and purification of these compounds. So, if we move towards keratin based bioplastics, we would be deriving a plastic alternative that will have no clashing nutritional needs of the society and also, it will be the best way of utilizing the human waste generated.

References

1. Navina Ramakrishnan, Swati Sharma, Arun Gupta, Basma Yahya Alashwal: Keratin based bioplastic film from chicken feathers and its characterization, International Journal of Biological Macromolecules,111, 352-358, 2018.
2. Justin R. Barone, Walter F. Schmidt, N. T. Gregoire : Extrusion of Feather Keratin, Wiley InterScience, DOI 10.1002/app.23501, 2005.
3. YaoDou,BuningZhang,MingHe,GuoqiangYin,YingdeCui: The structure, tensile properties and water resistance of hydrolyzed feather keratin-based bioplastics, Chinese Journal of Chemical Engineering, Volume 24, Issue 3, 415-420,2016.
4. Swati Sharma, Arun Gupta, Ashok Kumar, Chua Gek Kee, Hesam Kamyab,Syed Mohd Sauf: An efcient conversion of waste feather keratin into ecofriendly bioplastic flm, Clean Technologies and Environmental Policy, Volume 20, Issue 10, pp 2157–2167, 2018.
5. H.K. Ahn, M.S. Huda, M.C. Smith, W. Mulbry, W.F. Schmidt, J.B. Reeves III : Biodegradability of injection molded bioplastic pots containing polylactic acid and poultry feather fiber, Bioresource Technology, Volume 102, Issue 7, Pages 4930-4933,2011.

6. Oluwasina Olugbenga Oladayo1, Umunna Queendaline C. , Olusegun Sunday Joseph and Wahab Oluwasegun : Physicochemical properties of cassava starch and starch-keratin prepared biofilm, Songklanakarin J. Sci. Technol. 38 (4), 349-355, Jul. - Aug. 2016.
7. Peter M. M. Schrooyen, Pieter J. Dijkstra, Radulf C. Oberthu¨r, Adriaan Bantjes, and Jan Feijen: Partially Carboxymethylated Feather Keratins. 2. Thermal and Mechanical Properties of Films, J. Agric. Food Chem., 49, 221−230, 2001.
8. Angela Edwards, David Jarvis, Tracy Hopkins, Sarah Pixley, Narayan Bhattarai: Poly(ε-caprolactone)/keratin-based composite nanofibers for biomedical applications, Journal of Biomedical Material Research, Volume103, Issue1, 21-30, 2015.
9. K. Mohanty,P. Tummala, W. Liu,M. Misra,P. V. Mulukutla and L. T. Drzal: Injection Molded Biocomposites from Soy Protein Based Bioplastic and Short Industrial Hemp Fiber, Journal of Polymers and the Environment, Vol. 13, No. 3, July 2005.
10. Borja Fernández-d'Arlas: Tough and Functional Cross-linked Bioplastics from Sheep Wool Keratin, Scientific Reports volume 9, Article number: 14810, 2019.
11. Basma Y.Alashwal, Mohamed Saad Bala, Arun Gupta, Swati Sharma, Puranjan Mishra: Improved properties of keratin-based bioplastic film blended with microcrystalline cellulose: A comparative analysis, Journal of King Saud University – Science, 2019.
12. Shuai Liu, Kaibing Huang ,Han Yu , Fenxia Wu: Bioplastic based on 1,8-octanediol-plasticized feather keratin: A material for food packaging and biomedical applications, Inc. J. Appl. Polym. Sci., 135, 46516, 2018.
13. Silviana; Zuhri, Latif Alfiyan; Muzakki, Mukti Mabrur; Rahmawati, Adisty Kurnia; Marthasari, Annisa Ayu: Advanced Science Letters, Volume 23, pp. 5739-5741(3), Number 6, June 2017.
14. Kush Kumar Nayak, Piyush Parkhey, Bidyut Mazumdar:Keratin as a Protein Biopolymer, Keratin as a Protein Biopolymer, 201-224, 2018.
15. DianaGómez-Heincke,InmaculadaMartínez, Mats Stading, CríspuloGallegos, PedroPartal : Improvement of mechanical and water absorption properties of plant protein based bioplastics, Food Hydrocolloids,Volume 73, Pages 21-29, 2017.

16. Victor Perez, Manuel Felix, Alberto Romero, Antonio Guerrero: Characterization of pea protein-based bioplastics processed by injection moulding, food and bioproducts processing 9 7, 100–108, 2016.
17. S. Gamero, M. Jiménez-Rosado, A. Romero, C. Bengoechea, A. Guerrero: Reinforcement of Soy Protein-Based Bioplastics Through Addition of Lignocellulose and Injection Molding Processing Conditions, Journal of Polymers and the Environment, Volume 27, Issue 6, pp 1285–1293, June 2019.
18. Lucía Fernández-Espada, Carlos Bengoechea, Felipe Cordobés, Antonio Guerrero: Protein/glycerol blends and injection-molded bioplastic matrices: Soybean versus egg albumen, Journal of Applied Polymer Science, 133, 42980, 2016.
19. "Report on the Indian Plastic Industry". PlastIndia Foundation. January 2018.
20. Nakamura, A., Arimoto, M., Takeuchi, K., and Fujii, T.: A rapid extraction procedure of human hair proteins and identification of phosphorylated species. Biol. Pharm. Bull., 25, 569-572, 2002.
21. BENDEK, T. :Fragmente mycologia. Some historical remarks on the development of "hair baiting" of Toma-Karling-Van breusegham (To-ko-Vo hair baiting method). Mycopathol. Mycol. Appl. 16, 104-106, 1962.
22. Mariana Călin, Diana Constantinescu-Aruxandei, Elvira Alexandrescu, Iuliana Răut, Mihaela Badea Doni, Melania-Liliana Arsene, Florin Oancea, Luiza Jecua and Veronica Lazăr : Degradation of keratin substrates by keratinophilic fungi, Electronic Journal of Biotechnology, Volume 28, Pages 101-112, July 2017.
23. Vipul Agarwal, Arpana Gopi Panicker, Sushma Indrakumar, Kaushik Chatterjee : Comparative study of keratin extraction from human hair, International Journal of Biological Macromolecules, Volume 133, Pages 382-390, 2019.
24. Lutz Langbein, Michael A. Rogers,n Silke Praetzel, HermelitaWinter,n and J˙rgen Schweizern: K6irs1, K6irs2, K6irs3, and K6irs4 Represent the Inner-RootSheath-Speci¢c Type II Epithelial Keratins of the Human Hair Follicle, The Journal of Investigative Dermatology , Vol. 120, No. 4 April 2003.

25. Parry DA, Strelkov SV, Burkhard P, Aebi U, Herrmann H: Towards a molecular description of intermediate filament structure and assembly. Exp Cell Res 313(10):2204–2216, 2007.

26. Ajello, L., L.K. George: In vitro hair cultures for differentiating between atypical isolates of Trichophyton mentagrophyfes and Trichophyton rubrum Mycopathol. Mycol. Appl. 8,3-17, 1957.